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THE GENETIC STUDY ON NON-HODGKIN'S LYMPHOMA LARGE B CELL

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Abstract:

The Non-Hodgkin's Lymphoma Large B Cell (LBDGC) is a cancer of the lymphatic system that develops when an error occurs in the production of lymphocytes leading to the production of abnormal cells. Several genes govern the occurrence of NHL; one of the most widespread is the β-catenin one. This is why we tried in this study to evaluate the β-catenin gene effect on the induction of Non-Hodgkin's Lymphoma Large B Cell.

Keywords: lymphoma; β-catenin; DNA; cancer.

Introduction

Lymphoma is one of the most frequent homeopathies that exist; it is a cancer of the lymphatic system that develops when an error occurs in the production of lymphocytes leading to the production of abnormal cells who can proliferate in two ways: by dividing faster and / or living longer than normal lymphocytes (Add references). (Guide d'information, 2011).

Cancerous lymphocytes, such as healthy lymphocytes, develop in various parts of the body, especially in the organs of the lymphatic system such as lymph nodes, spleen, bone marrow, but also in all other organs; there are two main types of cancers of the lymphatic system:

- Hodgkin's lymphoma or Hodgkin's disease (LH).
- Non-Hodgkin's lymphoma (NHL).

Diffuse large-cell B lymphoma (LDGCB) is the most common type of NHL; when viewed under the microscope, the B cells of the lymphoma appear to be very large compared to normal lymphocytes. Moreover, it is noted that the LDGCB originates in the lymph nodes, but it can also be done in organs or tissues outside the lymph nodes. (Guide d'information, 2011).

The LDGCB increases by 10 to 20% every 5 years, representing the highest growth rate and the highest percentage of malignant homeopathies (18%), knowing that it affects more men than women with a sex ratio of 60% and 40% (Sylvain CHOQUET, 2007).

Several genes including the β catenin on which our genetic study was based on govern the occurrence of the NHL. The CTNNB1 is the β-catenin gene which is located on exon 3 and whose role is Proliferation During

development. B-catenin is primarily involved in proliferation in colon carcinoma cells; β-catenin has the ability to activate transcription of the cytotoxic proto-oncogene D1.

Apoptosis.

Inactivation of the CRD-BP (Coding Region Determinant-Binding protein) RNA-binding protein (Ribonucleic acid), which is a transcriptional target of β-catenin, results in induction of apoptosis. It is interesting to make the study of other gene whose their products has an interaction with the beta catenin protein. It is also interesting to see if there is a polymorphic site at the level of other genes.

Materials and methods

The study is part of a case-control study on NHL large B cell on a population of West Algeria (Oran-Tlemcen) with a sex ratio of 7 Men and 6 Women for the cases and 15 Men / 14 Women for Witnesses.

Sample

Blood samples were drawn on EDTA tubes (Ethylenediaminetetraacetic acid), on NHL patients with signed consent. DNA (Deoxyribonucleic acid) extraction was carried out in two methods Extraction of the DNA by NaCl (Sodium chloride*)*

The study is done in several stages: Thawing 20 or 30 ml (milliliter) blood at 70 °C (Celsius). Add TE (Tris EDTA) 10 / 10 to 45ml. Centrifuge at 2500 rpm (Revolutions per minute) for 15min (minute). Elimination of the supernate then add the TE10 / 10

Put the tube in ice for 10min and centrifuge at 2500 rpm for 15min. Repeat this step until a whitish pellet is obtained. At the lymphocyte pellet, add 5ml of lysis solution and 125 of proteinase K to 20mg (milligram) / l and homogenize the pellet. Incubation at 37 ° C overnight in a bain marie with gentle agitation. Addition of 2 ml of NaCl, vigorous stirring and centrifugation at 4000 rpm for 10 min. Recovery of the supernatant in another tube, add 2 volumes of absolute cold ethanol, let precipitate the DNA by turning the tube.

Recovery of the jellyfish by a sealed Pasteur pipette, rinse it once with 70% ethanol, place it in an eppendorf tube and let it dry in the open air. Dissolve the jellyfish in 200-500 of TE10 / 1. For complete dissolution, allow the tubes to stir slowly at room temperature for at least 24 hours.

Extraction of DNA by Maxwell® 16.

The automated steps of the Maxwell® 16 include: Binding of nucleic acids to paramagnetic particles. Washing of nucleic acids bound to the particles to separate them from other cellular components and DNA elution.

The proportioning of the DNA

Spectrophotometry

The DNA concentration is estimated by measuring the absorbance at 260nm (Nanometer). Into a quartz tank, distilled water is injected, then placed in the tank in the Spectrophotometer. In the second stage, the tank is withdrawn and a quantity of distilled water will be replaced by DNA, the whole will turn over in the spectrophotometer in order to visualize the result.

Amplification of β-caténin by PCR (polymerase chain reaction).

PCR RFLP (restriction length fragment polymorphism).

A PCR was carried out in order to amplify the fragments of gene CTNNB1 determined by starters using Taq (Thermus aquaticus) polymerase. The preparation of the reactional medium required a conical tube into which we inject distilled water, Master Mix, the 2 primers and the DNA.

The contents of the conical tube are divided on the PCR tubes, and then the DNA of each patient/ case is injected in the corresponding tube. The PCR tubes are put in the Thermal cycler then the operation is launched according to a process of 39 cycles which lasts 2h12 S(second) with the following stages Initialization denaturation Annealing elongation

Amplification Test.

An electrophoresis has been done with an agarose gel at 02%. The gel is prepared by dissolving the agarose powder in the buffer (TBE) ((Tris/Borate/EDTA)) using the micropipette, after homogenization, a comb is placed in the cast to create wells then the prepared gel is poured in the electrophoresis cast .The solidification of the Agarose gel requires 30 minutes approximately.

The buffer

The used buffer is Bromophenol Blue, which is used in order to sink the DNA sample in the bottom of the well. After the gel solidification, we pour the buffer so that the whole of the cast is immersed by it.

Loading of samples

After having mixed the DNA samples with the buffer, the samples are then charged in each well carefully to avoid any contamination. A suitable marker is also included in one of the wells, in order to estimate the size of the fragments of DNA samples.

Electrophoresis

The Device of electrophoresis is prepared by checking that the sons are directed in the good sense so that the sample migrates starting from the (cathode) to (anode).Once the adequate voltage is put, the electrophoresis proceeds for a large period that should allow a sufficient separation of the fragments, but not as a long as they thorough at the other end of the gel and be lost in the electrophoresis tank.

The digestion with BanI

Préparation of reactional medium

Reactional volume for simple digestions includes: ADNmt; Restriction endonuclease (Ban I), The buffer (depends on the restriction endonuclease) and distilled water. The reactional medium contained in a micro tube, was incubated at the enzyme incubation temperature 37°C for Ban I during 20 hours. The restriction sites for Ban I are:

5’ ⋯G▼G(T/C)(A/G)C C…3’

3’…C C(A/G)(T/C)G▲G⋯5’

These measurements were done for 40 samples: 27 witnesses and 13 cases and it resulted a very clear band because of the weak concentration of DNA, which was 5µL (microliter).

Result

After the extraction of the DNA, we have noticed that the quality of the jellyfish of the DNA obtained by the kit is better than that of the NaCl technique. Once the spectrophotometry was done, it has been followed by an amplification of the patients DNA, which resulted a band of 157bp (base pair).The last step was the DNA digestion and it has showed an almost similar size of the bands at the cases and witnesses. Ajouter la photo du gel après l’utilisation de l’enzyme de restriction

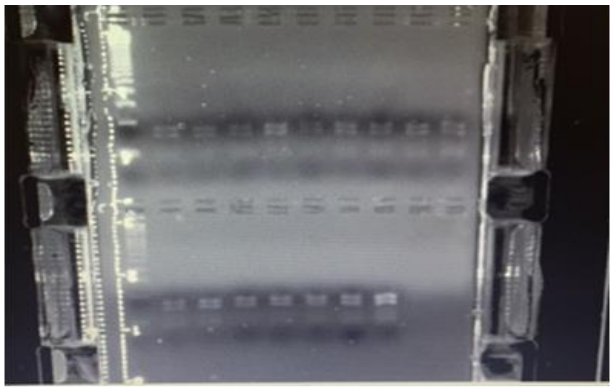

Figure 1. Result of digestion with the enzyme BanI.

Discussion

The lymphoma is a malignant blood disease which touches cells of the immune system B and T, the epidemiological studies showed that the diffuse lymphoma with large cells B (LDGC-B) is the most frequent lymphoma, accounting for approximately 40% of the whole of the lymphomas. This justifies the many studies conducted to elucidate the etiology of this pathology and its mechanism of development, by various methods of studies: CAS-witness, Troops, and even of a fundamental and molecular nature. The diffuse lymphoma with large cells B (LDGC-B) is multifactorial and multigenic. Epidemiological studies of association made it possible to put in obviousness some risk factors who are linked to each study and in different ethnicity such as UV radiation, some immunosuppressor drugs.

Other association studies try to highlight some candidate genes, which could be responsible for the release and the development of the process tumorigeneses of the LNH and especially for the LBGC. The polymorphism study on the CTNNB1 gene for the beta catenin protein is considered as a tumor suppressor. This study lies within the scope of a study CAS-Witnesses on LNH, aimed to index the polymorphic sites on the level of gene CTNNB1 located on the level of the exon 3. The results revealed that the studied site, in ours population cannot be regarded as polymorphic since the results of the genetic study did not show any difference in the genotypic profiles between all the subjects studied case and witnesses. Therefore, this site cannot be considered as genetic marker.

Conclusion

After the study that one carried out on the diffuse LNH large cells B and gene CTNNB1 we have obtained the same profile of digestion among patients and witnesses. That is what enabled us to conclude that the gene is not polymorphic. The study by other methods of molecular genetics like as the expression of transcribed and using RT-PCR (Reverse transcription polymerase chain reaction) method. Moreover, It is interesting to make the study of other gene whose their products has an interaction with the beta catenin protein.

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Ethics

Blood samples were adjustments on patients who signed informed consent

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